ON THE NATURE OF IMMUNITY AGAINST PLAGUE

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By:

U. S. DEPARTMENT OF COMMERCE
OFFICE OF TECHNICAL SERVICES
JOINT PUBLICATIONS RESEARCH SERVICE
Building T-30
Ohio Drive & Independence Ave., S.W.
Washington 25, D. C.

ON THE NATURE OF IMMUNITY AGAINST PLAGUE

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From the "Mikrob" All-Union Scientific Research Institute

It has been established that plague disease is characterized by a slow buildup of immunity; only in exceptional cases does the organism successfully overcome this infection.

Experiments on the immunogenesis of live vaccine have shown that guinea pigs and white mice build up resistance against plague during the period of the vaccinal process and in the first days following inoculation, when live microbes are still present in the organs and tissues of the animals (Korobkova, 1956; Korobkova, Gorokhov, Sivolyubov, Lobanov, 1960; Aliyev, 1959; Samoylova, 1961). At the same time, a study of the dynamics of the formation of anti-plague immunity showed unequivocally that with the liberation of the organism from vaccinal microbes, the immunity is fully retained. These studies indicated the possibility of sequential phases in the development of immunity against this infection.

Until recently, the notions of the nature of anti-plague immunity were limited to the recognition of solely sterile immunity which arises after the overcoming of the infection or after vaccination. With the accumulation of experimental data, especially as a result of the study of immunity following after the inoculation of animals with vaccine, there arose the concept of the existence of a non-sterile (infectious) immunity phase which then passes into sterile (postinfectious) immunity after the organism is free of live microbes.

Such an understanding of anti-plague immunity justifies the impossibility of reproducing stable and prolonged immunity with the aid of killed microbes and their antigen fractions.

The fact that certain animals (mice, rats) can be protected from infection by repeated injections of sorbed killed vaccine (Spivak and coauthors, 1958) and large doses of chemical fractions (fraction 1) extracted from the plague microbe, and thus immunized, does not contradict the supposition that live vaccine is not the most effective preparation capable of producing durable and intensive immunity under experimental conditions (in guinea pigs -- up to 13-14 months). At the present time the problem of the effectiveness of killed plague vaccines is being reexamined from the standpoint of the immunogenicity of individual

chemical fractions of the plague microbe, the repetition of vaccination, and the addition of sorbed killed vaccines to the antigen.

According to Meyer and associates (1955), the immunogenicity of live or killed plague vaccine is determined by its content of fraction 1. A well-purified fraction immunizes mice, rats, and monkeys, but not guinea pigs. Spivack and associates (1958) attempted to solve the problem of immunizing guinea pigs. According to their data, killed plague microbes suspended in a physiological solution are ineffective in protecting guinea pigs from plague, but killed microbes suspended in oil are a means of immunizing these animals. The method of plague microbe killing (phenol, formalin, alcohol) is immaterial. On this basis the authors concluded that there is no destruction of antigens essential to immunogenesis in the killing process. However, Korobkova (1935) found that killed plague microbes sorbed in oil (lipovaccine) did not protect guinea pigs from infection, but merely prolonged the life of infected animals in comparison with the control group.

Contrary to the indications of Otten (1936), Jawetz and Meyer (1943), Spivack and associates did not detect an immunogen in the plague microbe which was specific to guinea pigs; fraction 1, which is a protective antigen for guinea pigs, likewise immunizes mice, rats, and monkeys. The disagreement in findings, according to Spivack, stems from the fact that the other researchers had used excessively large doses of fraction 1. The introduction of a 1 mg dose of fraction 1 into guinea pigs no longer protects them from infection, but leads to the inhibition of immunogenesis; however, the administration of preparations containing merely trace amounts of fraction 1 produces the production of antibodies to fraction 1 by the organism, which does provide immunity.

The results of Spivack and associates were not confirmed by Soviet researchers (Drozhevkina, Tinker, Levi, et al., 1961; Faybich and coauthors, 1961). In the experiments of Drozhevkina and associates, small doses of fraction 1 (0.2-1 μ g) not only failed to prevent the death of guinea pigs, but led to more rapid death of the inoculated animals in comparison with those in the control group, while guinea pigs immunized with live microbes of the EB strain survived 100% under the same infection conditions. The same conclusions were drawn by Faybich and coauthors, who found that fraction 1 (0.1 mg) administered separately and in combination with the live EB culture inhibits rather than stimulates immunogenesis. Large doses of fraction 1 protect only mice.

Thus, the problem of which antigen is needed to protect man from plague is as yet unclear.

Meyer and Foster (1948) used volunteers to study the possibility of antibody formation upon the introduction of fraction 1, a vaccine containing virulent microbes killed with formalin, the A1122 avirulent strain, and the Otten strain. Franction 1 and the killed vaccine were introduced in three large doses; the live vaccine (1 billion microbes) was introduced in a single dose. The result was that the serum of the volunteers inoculated with fraction 1 (2.5 mg) was found to contain preventive antibodies for mice. The serum of those inoculated with the

formalin vaccine and the A1122 live culture, such antibodies appeared in low titers. The Otten strain turned out to be least active. According to the data of Smith and coauthors (1960) the effectiveness of fraction 1 for mice is not higher than the effectiveness of killed corpuscular vaccine. Until the present time, there have been no observations of the epidemiological effectiveness of chemical vaccines. According to all data, chemical as well as killed vaccines provided immunity of relatively large strength and duration. Even two inoculations with large doses of fraction 1 adn killed vaccines were inferior in effectiveness to injections of live vaccine (Table 1).

Many researchers are inclined to think that man in his sensitivity to plague is more like the mouse than the guinea pig, inasmuch as man, as the mouse, is highly sensitive to the plague toxin, while the guinea pig is only very weakly sensitive to it. This assertion, of course, applies to the toxin produced in the test tube. The guinea pig is indeed resistant to such a product. But this understanding of the toxin is highly limited. The guinea pig infected with virulent plague microbes perishes from products released by an enormous number of microbes poisoning the organism, i.e., in the final analysis, from the toxin.

Although the advocates of chemical vaccines attach great importance to fraction 1 extracted from the plague microbe, they do not deny the superiority of live vaccines. In order for fraction 1 to provide even mice and rats approaching the level of the immunity provided by live avirulent immunogenic microbes, it must be administered repeatedly and in large doses. Immunogenic effectiveness is likewise improved by the use of sorbed vaccines.

The problem of factors determining the immunogenicity of live vaccine was studied in a number of experiments by Burroughs (1960) who showed that the immunogenic vaccinal strains EB et al., contain V- and W-antigens and franction 1 (Fl+), just as virulent strains.

In addition, it has been proved that immunogenic vaccinal strains reproduce in the organism of the subject and produce hyperplasia of cell elements of the lymphoid organs which are the basic producers of antigens.

Such producer-cells are the plasmocytes formed from the reticular cells.

In the cytomorphological study of animals immunized with live vaccine, the lymphoid organs reveal a plasmocytaric reaction manifested in the accumulation of plasmocytes with a basophilic cytoplasm. A necessary stage in plague immunogenesis is the penetration of vaccinal microbes into plasma cells. This process sensitizes the cells, imparting to them a heightened reactivity in subsequent encounters with the antigen.

Such changes cannot be observed upon injection with killed virulent or avirulent plague microbes and various fractions. Stable active immunity against plague can be produced with live immunogenic strains with reduced virulence, since the mere presence of live microbes in the vaccine does not yet determine its effectiveness. Avirulent plague microbes without specific antigens (Vi-antigen) which

have lost the ability to reproduce and remain in the animal's organism, there producing changes characteristic of the vaccinal process, do not produce anti-plague immunity.

The intensity of semination of internal organs with vaccinal microbes and their topographical distribution within these organs may differ depending on the animal and strain, but both factors are necessary for the production of immunity. Thus, in the organism of the white mouse, immunogenic vaccinal strains, injected subcutaneously in a 50-100,000 microbe dose reproduce well, and for a period of 10-18 days they can be observed in specimens taken not only from the point of introduction, lymph nodes, spleen, and liver, but also in the blood and lungs (Korobkova, 1956; Aliyev, 1959, Samoylova, 1961). In guinea pigs, on the other hand, even after the subcutaneous injection of 3-5 billion microbes, the vaccinal strains are mostly detectible at the point of introduction, the regional lymph node, and spleen, and more rarely in the liver, bone marrow, and in exceptional cases, in the blood (single colonies), and even then upon autopsy after 3-4 days. They remain for 10-15 days at the point of introduction, the lymph node, and the spleen. Common to all animals sensitive to plague is the selective settling of microbes in the lymphatic and hemopoietic systems.

And so, the basis of the reproduction of stable anti-plague immunity is the phase of non-sterile immunity. Active immunity develops parallel to the vaccinal process. Non-sterile immunity in its further development becomes more intense and passes into sterile postinfectious immunity.

Postinfectious anti-plague immunity is characterized by its dependence on the duration and intensity of the non-sterile immunity phase, especially for guinea pigs. Experiments on many avirulent strains of the plague microbe have shown that their brief retention in the organism of the inoculated animal led to a weak immunity in both phases (Korobkova, 1956; Aliyev, 1959).

Proof of the dependence of immunity on the intensity of organ insemination by plague microbes is provided by the weak immunity or even the total lack of immunity in guinea pigs, and to a lesser extent, in white mice surviving after infection with virulent plague microbes as a result of streptomycin treatment. In such cases, with repeated infection with virulent plague microbes, the recovered animals die along with the control specimens (Table 2). In addition to the fact that streptomycin inhibits immunogenesis, there is a direct connection between the bacterial action of the antibiotic and the absence of a non-sterile immunity phase (Korobkova, 1946; Samoylova, 1961). Humans treated with streptomycin likewise do not acquire anti-plague immunity. This is indicated by the negative intradermal reaction to pestine (Kozlov and coauthors, 1958). The quickly-passing infection, which does not produce sterile immunity phenomena, does not leave behind any resistance to infection, and the second sterile immunity phase fails to ensue.

Moreover, in the case of a disruption of the normal immunogenetic cycle as a result of streptomycin administration in the first hours following immunization of mice and guinea pigs with live EB-strain

cultures, the vaccinal microbes perish and immunity does not develop (Table 3). Only that group of animals survived which received streptomycin 3 days after injection, when the vaccinal process has already begun to develop.

According to the cited interpretation, the development of immunity in the case of plague is analogous to its development in tularemia and brucellosis. The transition of the non-sterile immunity phase into the sterile one is characterized by a change in the immunological reactivity of the mesenchymal tissues with respect to the exciting agent, the accumulation of metabolites which inhibit the biosynthesis of the plague microbe, and the increased role of barrier-fixing protective mechanisms in the host.

From the standpoint of the humoral theory of immunity, the advantage of live vaccines over killed ones is groundless, in particular with regard to antibody production. The serum of animals repeatedly injected with killed microbes reveals hemagglutinines and complement-binding substances, although they perish upon infection with a large dose of virulent plague culture. At the same time, animals immunized once with live immunogenic vaccine, despite the fact that their serum does not reveal the indicated antibodies, turn out to be resistant to infection.

However the problem of the factors in acquired immunity might eventually be solved, there can be no doubt that it is based on the non-sterile phase which determines the level of sterile immunity in most species of animals.

Conclusions

1. As a result of the study of immunity developed after injection with live plague vaccine, and experimental basis has been provided for the theory of the presence of a phase of non-sterile immunity which is supplanted by sterile immunity after the organism has eliminated the vaccinal microbes. A special characteristic of sterile immunity is its dependence on the duration and intensity of the non-sterile immunity phase (vaccinal process).

2. Killed chemical vaccines of various types create a rapidly diminishing immunity of relative intensity. The immunogenicity of various fractions of the plague microbe is considerably inferior to the immunogenicity of live vaccine prepared from immunogenic strains. The effectiveness of chemical vaccines has in addition not yet been confirmed by epidemiological observations.

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Table 1

Immunogenic Properties of EB Live Avirulent Culture and Killed Antigens (infection with 100 Dcl)

Antigen	Dose (in microbes)	No. of inoculations	No. of mice in experiment	No. surviving	
				Absolute	%
*EB NIIEG	10 000	Single	24	18	75
EB-12	10 000	"	25	22	88
EB-13	10 000	"	25	20	80
Killed	150 000	"	25	2	8
vaccine	100 000	"	25	2	8
	50 000	"	15	0	0
Fraction 1	1.6 mr	Double	25	14	56
Control	---	---	25	0	0

*(Nauchno-Issledovatel'skiy Institut Epidemiologii i Gigiyeny -- Epidemiology and Hygiene Scientific Research Institute)

Table 2

Results of repeated Infection With Plague Microbes of Guinea Pigs Cured of the First Infection by Streptomycin (from data of Korobkova, Samoylova, and Nechetskaya)

Type of animal	Time between first infection and start of treatment (hours)	Time of repeated infection after cure (days)	Infection dose (Dcl)	Number of animals with repeated infection	Number of animals killed	Number of control animals with a second infection	Number of animals killed
Guinea pigs	12	42	300	4	4	5	5
	20	42	300	6	6	5	5
	24	30	100	15	15	5	5
	24	180	150	17	17	5	5
	48	210	150	14	14	5	5
	48	35	200	20	19	18	18
	72	36	200	8	8	5	5
	24	25	200	8	8	4	4
White mice	24	25	40	26	17	12	12
	48	35	80	25	20	15	15

Table 3

Testing of Immunity in Mice and Guinea Pigs Treated with Streptomycin at Various Times Following Vaccination

Type of animal	Time of streptomycin administration following injection (hours)	Injection dose	Infection dose (Dcl)	Results of infection 30 days after inoculation	Effectiveness index
White mice	3	20 000 microbes	50	0/9	18
	6			0/9	18
	12			0/9	20
	24			1/9	18
	48			2/9	14
	72			4/9	9
	96			6/9	6
	Untreated inoculated specimens			10/10	
	Unvaccinated control specimens			0/10	32
Guinea pigs	24	500 000 microbes	200	0/10	20
	48			1/10	14
	Untreated inoculated specimens			10/10	
	Control specimens			0/10	16

Note: numerator -- number of surviving animals; denominator -- number of animals in experiment.

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